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Short communication

Heterostyly in *Nymphoides indica* (Menyanthaceae) in Japan

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Nymphoides indica (L.) O. Kuntze (Menyanthaceae) is a perennial, floating-leaved, heterostylous, aquatic plant. In this paper the dimorphism of the flowers including differences in the distance between staminate and carpellate parts and differences in pollen size between long-styled and short-styled floral morphs is documented. Seed set was observed only in intermorph pollinations. *Nymphoides indica* was confirmed to be heterostylous, herkogamous and self-incompatible.

Key words: floral dimorphism, herkogamy, heterostyly, *Nymphoides indica*

Nymphoides (Menyanthaceae) is a cosmopolitan genus of 20 species of floating-leaved aquatic plants (Cook 1990). The various species show different reproductive systems, including dioecy, heterostyly, partial homostyly or a breakdown in heterostyly, and autogamy (Ornduff 1966; Barrett 1980; van der Velde & van der Heijden 1981; Marui & Washitani 1993).

Nymphoides indica (L.) O. Kuntze is a distylous, perennial plant (Ornduff 1966, 1970; Sculthorpe 1967). Reddy and Bahadur (1976) and Barrett (1980) investigated some traits of heterostyly in populations of *N. indica* in India and Brazil, respectively, and showed that intermorphic pollination is usually required for fruit and seed set. In Japan, *Nymphoides indica* grows in meso- to eutrophic lakes and ponds, mainly in the southwest (Kadono 1994). Hamashima (1979) observed fruit and seed set in *N. indica* in the Tokai Region, central Japan, and showed that floral morph composition influenced seed set. The purpose of our study was to reevaluate features of heterostyly in *N. indica* in Japan with special emphasis on quantitative aspects.

To confirm dimorphism in the flowers of *Nymphoides indica*, we measured the length of the stamens and pistils in two floral morphs – long-styled and short-styled. The flowers of both morphs were sampled in seven populations, as shown in Table 1. The floral morph composition in populations HB, HC, HE and HO (see Table 1) was so biased that we sampled only the dominant floral morphs. Twenty flowers (one per inflorescence) were collected from each population and fixed in FAA (formalin, acetic acid and ethanol) in the field. The length of the stamens and pistils were measured to 0.1 mm with an ocular micrometer. The five stamens in each flower were nearly equal in

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TABLE 1. Localities of populations investigated.

Locality (Population code)
Hyogo Prefecture
1. Kande-cho, Nishi-ku, Kobe City (HB)[a]
2. Hiraoka-cho, Kakogawa City (HC)
3. Higashikanki-cho, Kakogawa City (HD)
4. Nishikanki-cho, Kakogawa City (HE)
5. Heiso-cho, Kakogawa City (HG)
6. Ouji-cho, Ono City (HI)
7. East, Aono-cho, Kasai City (HO)
8. Kuchisami, Hayashida-cho, Himeji City (HV)

a) Boldface indicates populations where SCD (Staminate-carpellate distance) was investigated.

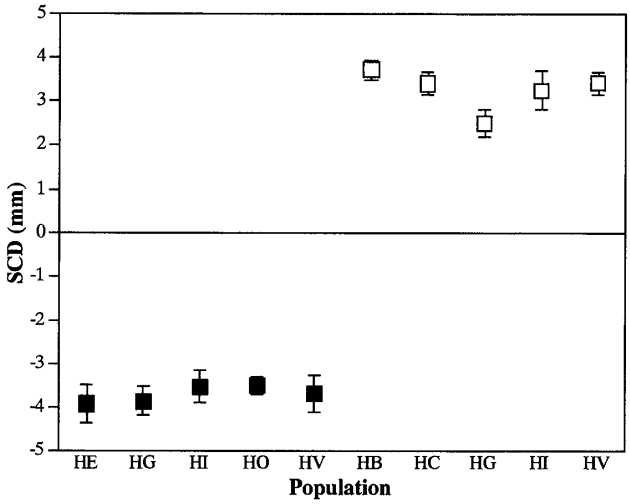

FIG. 1. Staminate-carpellate distance (SCD) of long-styled (closed) and short-styled (open) morphs collected from seven populations. The mean and SD are shown. For population codes, see Table 1.

length, so we measured one stamen per flower. The length of the stamen and pistil was measured from the base of the ovary to the top of them. The difference between these two lengths is referred to as the staminate-carpellate distance (SCD) (the "MFD(Male-female distance)" of Marui & Washitani 1993).

The staminate-carpellate distance (SCD) of both morphs in seven populations is shown in Fig. 1. The differences in length of the pistil and stamen were clear in both morphs. The differences show a clear bimodal distribution, thereby confirming the existence of the two floral morphs.

To compare pollen size between long-styled and short-styled morphs, we collected flowers of each morph from cultivated plants that originated from the study populations labeled HC and HD in Table 1. Twenty flowers each were sampled from the long-styled morph in population HD and the short-styled morph in HC. The pollen grains were gathered at anthesis with fine forceps and placed on a glass slide in a 70% alcohol solution. The diameter of 400 grains from the long-styled morph and 600 grains from the short-styled morph were measured in photomicrographs.

Figure 2 shows the frequency distribution of pollen size for both morphs. The grain diameter was 31.7 ± 1.5 μm (range: 27.5-35.8 μm; N = 400) in

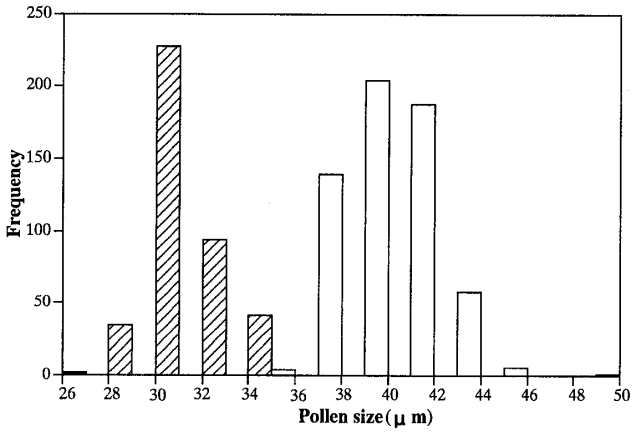

FIG. 2. Comparison of pollen size between long-styled (hatched) and short-styled (open) morphs.

long-styled flowers and 39.6 ± 2.0 μm (range: 35.5-48.2 μm; N = 600) in short-styled flowers. The difference in pollen size between the two morphs was about 8 μm.

To determine the compatibility between the two morphs of *Nymphoides indica*, we conducted pollination experiments from 15 to 30 August 1998 using cultivated plants. We compared the following three treatments: 1) reciprocal pollination between the two morphs; 2) intramorph pollination between different plants of the same morph; and 3) self-pol-

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lination. Flowers used for cross pollination were emasculated in the bud with fine forceps on the day before opening. Both long-styled and short-styled morphs were used as maternal and paternal parents in reciprocal crosses in the three treatments. The number of flowers ranged from 17 to 22 for each treatment. Crossing was conducted from 8:00 a.m. until 12:00 at noon. After pollination, the whole plant was covered with semi-transparent cloth to exclude pollinators. We assumed that photosynthesis was not significantly reduced, since the cloth reduced radiation by only 10%.

The fruits were collected after three weeks (when they had matured). Fruit set rate (number of fertile fruits / number of pollinated flowers) and seed set rate (number of mature seeds / total number of ovules) were calculated using a stereomicroscope.

The results of the pollination experiments are shown in Table 2. In cross pollination between long-styled and short-styled morphs the average seed set rate was 0.74 in long-styled and 0.76 in short-styled morphs. No seeds resulted from intramorph pollinations or from self-pollinations in either morph.

Our study showed a bimodal distribution of the SCD corresponding to long-styled and short--styled floral morphs in *Nymphoides indica*, indicating that spatial separation of the stigma and anthers in one morph is maintained in *N. indica*; in other words, there are no homostylous morphs. Pollen size in the two floral morphs was also confirmed to be different, as was reported by Ornduff (1966), Reddy and Bahadur (1976), and Hamashima (1979). The difference of 8 μm was roughly in accordance with the results of Ornduff (1966) and Hamashima (1979), but was different from those of Reddy and Bahadur (1976), who reported that the difference in pollen size was about 3.7 μm in Indian populations. The discrepancies may be due to differences in methods of measuring, but geographical variation in pollen size can also occur.

In pollination experiments, fruits and seeds resulted from only intermorph pollinations. Although Barrett (1980) reported that self-incompatibility in *Nymphoides indica* was somewhat incomplete, our observations indicate that self-incompatibility in Japanese populations is as strong as in populations in India (Reddy & Bahadur 1976). We further confirmed that self-incompatible heterostyly is maintained in the populations of *N. indica* in Japan. There was also no evidence of a breakdown in heterostyly in *N. indica*, as has been reported in *N. peltata* (Ornduff 1966; van der Velde & van der Heijden 1981; Marui & Washitani 1993).

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TABLE 2. Rate of fruit and seed set following artificial pollination in *Nymphoides indica*.

Treatment	Pollination attempts	Fruit set rate (%)	Seed set rate Mean ±SD (%)
Long-styled (Maternal)			
intermorph	17	100	74.3±25.2
intramorph	18	0	0
self	18	0	0
Short-styled (Maternal)			
intermorph	17	100	76.0±18.9
intramorph	18	0	0
self	22	0	0

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